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RESEARCH

Impact of systemic resveratrol on non-surgical periodontal treatment of smokers: A 12-month randomized clinical trial

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Abstract

Objective Smoking patients demonstrate an elevated risk of periodontitis development and respond poorly to periodontal therapy as compared to nonsmokers, and resveratrol (RSV) demonstrated a positive effect in the reduction of periodontitis progression in both animal and clinical trials. However, to the authors' knowledge, no clinical study has assessed the impact of resveratrol under smoking conditions. Thus, this trial aimed to evaluate the effect of systemic administration (SA) of RSV adjunct to full-mouth ultrasonic debridement (FMUD) of periodontitis smoking patients (PSP).

Materials and methods Thirty-eight individuals were randomly assigned to two groups:**Placebo ($n=19$)** –FMUD and placebo for 180 days; **RSV ($n=19$)** FMUD and RSV (500 mg/day) for 180 days. Clinical and immunoinflammatory outcomes were assessed at baseline, 3-, 6-, and 12-months post-therapy, and microbiological outcomes were evaluated at baseline, 3-, 6- months post-therapy.

Results RSV appeared to induce lower PD [2.96 (0.41) −3 months; 2.85 (0.40) – 6 months; 2.80 (0.35)- 12 months], CAL [4.02 (0.90) – 3 months; 4.04 (0.81) – 6 months; 3.87 (0.78) – 12 months], and PMG [2.20 (0.56) – 3 months; 2.28 (1.14) – 6 months; 2.32 (3.27) – 12 months] readings as compared to Placebo [PD: 3.22 (0.51) – 3 months; 3.07 (0.42) – 6 months; 3.02 (0.42) – 12 months; CAL: 4.43 (0.99) – 3 months; 4.24 ((0.89) – 6 months; 4.39 (0.93) – 12 months; PMG: 2.50 (0.50) – 3 months; 2.53 (0.45) – 6 months; 2.67 (0.46) – 12 months] throughout the time ($p<0.05$). The concentration of *Aggregatibacter actinomycetemcomitans (Aa)* was significantly higher in moderate [2.29 (1.10); 1.61 (1.02) for PL and RSV, respectively] and deep PD [2.39 (1.14); 1.73 (0.90) for PL and RSV respectively] at 3 months for the Placebo group ($p<0.05$). Additionally, Aa levels were lower at 6 months in the deep sites for the RSV group ($p<0.05$) [1.77 (0.94); 2.23 (1.08) for PL and RSV, respectively]. Immunoinflammatory analysis showed lower levels of IL-1β at 3-month periods in deep sites in the RSV group [92.6 ± 84.2; 35.36 (52.92) for PL and RSV, respectively] and lower concentrations of IL-6 in the RSV group at 3 and 12 months in both moderate [8.11 (9.50); 4.67 (4.20) – 3 months for PL and RSV, respectively; 8.01 ± 3.52; 5.33 (4.14) – 12 months for PL and RSV, respectively]; and deep sites [4.69 (3.06); 3.57 (3.73) – 3 months for PL and RSV, respectively]; 3.50 (2.67); 2.10 (0.89) – 12 months for PL and RSV, respectively] ($p<0.05$).

Conclusion In conclusion, systemic administration of RSV improves clinical results and modulates IL-1β at 3 months, IL-6 at 3- and 6- months, in deep sites of smoking patients when associated with FMUD.

Trial registration Rebec identifier https//ensaiosclinicos.gov.br/rg/RBR3gt65c.

Keywords Smoking · Periodontitis · Resveratrol · Periodontal debridement.

Extended author information available on the last page of the article

Clinical relevance

Scientific rationale for study: A higher risk of periodontitis development and poor response to periodontal therapy are consequences of smoking. RSV has demonstrated a positive effect in the reduction of periodontal breakdown in animal models, both in normal and other conditions such as smoking. Clinical studies have verified the effect of RSV supplementation during non-surgical periodontal treatment of healthy and diabetic patients, observing modulation of immunological markers and improvement of clinical parameters. Within the authors' knowledge, no clinical study has evaluated the effect of resveratrol under smoking conditions.

Principal findings: RSV group demonstrated significant improvements compared to the placebo group. Specifically, there were reductions in probing depth (PD) [2.96 (0.41) – 3 months; 2.85 (0.40) – 6 months; 2.80 (0.35) – 12 months], clinical attachment level (CAL) [4.02 (0.90) – 3 months; 4.04 (0.81) – 6 months; 3.87 (0.78) – 12 months], and plaque microbial growth (PMG) [2.20 (0.56) – 3 months; 2.28 (1.14) – 6 months; 2.32 (3.27) – 12 months] over time ($p<0.05$) when compared to the placebo group (PD: 3.22 (0.51) – 3 months; 3.07 (0.42) – 6 months; 3.02 (0.42) – 12 months; CAL: 4.43 (0.99) – 3 months; 4.24 (0.89) – 6 months; 4.39 (0.93) – 12 months; PMG: 2.50 (0.50) – 3 months; 2.53 (0.45) – 6 months; 2.67 (0.46) – 12 months). The concentration of *Aggregatibacter actinomycetemcomitans (Aa)* was significantly higher at 3 months in the placebo group in both moderate [2.29 (1.10) for PL and 1.61 (1.02) for RSV] and deep PD [2.39 (1.14) for PL and 1.73 (0.90) for RSV] sites ($p<0.05$). Moreover, Aa levels were significantly lower at 6 months in the deep sites for the RSV group (1.77 (0.94) for PL and 2.23 (1.08) for RSV at 3 months; 1.73 (0.90) for PL and 1.77 (0.94) for RSV at 6 months). Immunoinflammatory analysis showed lower levels of IL-1β at 3 months in deep sites for the RSV group [92.6±84.2 (PL) and 35.36 (52.92) (RSV)] and reduced IL-6 concentrations at 3 months in moderate [8.11 (9.50) (PL) and 4.67 (4.20) (RSV)] and deep sites [4.69 (3.06) (PL) and 3.57 (3.73) (RSV)], as well as at 12 months in both moderate [8.01±3.52 (PL) and 5.33 (4.14) (RSV)] and deep sites [3.50 (2.67) (PL) and 2.10 (0.89) (RSV)] ($p<0.05$).

The results demonstrated improved clinical outcomes, immunoinflammatory modulation with reduced pro-inflammatory markers, and a decreased count of *Aa*.

Practical implications: These findings confirm the hypothesis that resveratrol, as an adjunctive treatment, improves clinical outcomes, regulates inflammatory markers, and reduces periodontal pathogens in smokers undergoing non-surgical periodontal treatment. RSV is an interesting and safe bioactive agent that can be used as an adjunct to periodontal therapy, especially in smoking patients.

Introduction

Tobacco smoking is known as a risk factor for periodontitis [1, 2], and it is correlated with worse periodontal status [3, 4], higher plaque index, and calculus quantity [5]. There is evidence that exposure to cigarette smoke results in several effects related to the pathogenesis of periodontal disease in smokers, activating inflammatory cells and increasing levels of inflammatory markers [6, 7], as well as modifying patients' microbiome to a more pathogenic profile [8, 9]. Another modification observed in smokers is suppressed angiogenesis [10], the mechanisms of which are also under investigation. Additionally, smoking reduces the repair capacity of periodontal tissues by reducing cell function (fibroblasts, osteoblasts, and cementoblasts). These modifications lead to a reduced response to periodontal therapy in patients who are smokers. Several theories were suggested to explain the mechanisms related to the negative effects of smoking on periodontal tissues, and the most acceptable one is associated with the activation of the aryl hydrocarbon receptor and downstream effects on inflammation as well as oxidative stress [11, 12]. Smoking increases the local and systemic levels of metalloproteinases, which are related to tissue degradation [13], and increases saliva and serum levels of Receptor Activator of Nuclear Factor-κB Ligand/Osteoprotegerin (RANKL/OPG) in saliva and serum due to the reduction in the levels of OPG [14].

Host response modulation is widely studied as an alternative or adjunctive approach to managing periodontitis. Resveratrol (RSV), a phytochemical with anti-inflammatory, antioxidant, and other protective effects [15–20], has shown benefits in reducing periodontal breakdown in experimental models, including healthy and systemically compromised animals (e.g., smoking inhalation, diabetes, rheumatoid arthritis, and estrogen deficiency) by modulating inflammation and oxidative stress [21–27]. In smoking-related conditions, RSV acts as an aryl hydrocarbon receptor antagonist, counteracting smoke-induced effects, preventing osteoblast inhibition, and reducing reactive oxygen species (ROS) synthesis [28].

Some clinical studies evaluated the effect of RSV on periodontitis [29–31]. The first clinical study investigated the impact of 240 mg of resveratrol as an adjuvant to non-surgical periodontal therapy for 4 weeks in diabetic periodontitis patients. The authors observed reduced IL-6 levels for the RSV group without clinical improvement [29]. Javid et al. (2019) [29] also verified the effect of

resveratrol in patients with type 2 diabetes with periodontal disease. The patients received either 480 mg/day of RSV or placebo capsules for 4 weeks. RSV reduced the levels of fasting insulin resistance and the mean pocket depth. Zhang et al. (2022) [30] evaluated the effect of high, middle, and low doses of R RSV (500 mg, 250 mg, 125 mg) in aggressive periodontitis patients, observing a decrease in TNF-a, granulocyte-macrophage colony stimulating factor, macrophage inflammatory protein-1alpha (MIP-1a), fibrinogen, IL-2, CRP, INF-g, IL-1b, IL-8, IL-10 and IL-12p40. Nikniaz et al. (2023) [31] administered 480 mg/day of RSV for 4 weeks for periodontitis, without systemic conditions, in patients who received non-surgical periodontal therapy. However, they did not verify differences in clinical and immunological parameters.

Although the referred studies have shown some positive effects of RSV on the periodontal clinical parameters, and the pre-clinical studies have also shown an interesting effect of RSV in healthy and compromised systemic conditions, the impact of this substance in the presence of smoking has not been explored clinically. To the authors' knowledge, no clinical study has evaluated the effect of RSV on periodontitis smoking patients (PSP). In this context, considering the effects of RSV in animal models with epigenetic modifying factors such as smoking and the need for improvement in the response of smokers to periodontal treatment, the authors suggest that it is necessary to investigate the effect of RSV as an adjunct therapy for non-surgical periodontal treatment.

This study hypothesizes that systemic resveratrol, when used as an adjunct to non-surgical periodontal therapy in smokers, can enhance clinical parameters, regulate pro-inflammatory and anti-inflammatory markers, and decrease the number of periodontopathogens. Thus, this trial aimed to evaluate the effect of systemic administration (SA) of RSV adjunct to full-mouth ultrasonic debridement (FMUD) of periodontitis smoking patients (PSP).

Materials and methods

Study design

This study was a parallel, double-blinded, randomized clinical trial (Rebec identifier: RBR-3gt65c) performed in compliance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines. The study was approved by the university's review board (Ethics Committee of Paulista University—1.542.367) and complied with the 2013 revision of the 1975 Helsinki Declaration. The patients included gave their informed and written consent.

Population screening

Thirty-eight individuals were recruited from the patients who were referred to Paulista University. Patient recruitment was initiated in January/2015 and ended in December/2017. The clinical interventions and evaluations were from April 2016 to September 2018, and patient follow-up was finished by September 2019. Laboratory assays occurred between September and December 2020. By the end of September 2021, data entry and analysis were completed. The inclusion criteria proposed are as described below:

- Chronic periodontitis (Stage III or IV, Grade C periodontitis) [32], with at least eight teeth having periodontal pockets with PD and CAL≥5 mm with Bleeding on probing (BoP); at least 2 of these eight teeth should have PD and CAL≥7 mm.
- The oral cavity with a minimum of 20 teeth.
- A minimum of 10 cigarettes/day for at least five years of consistent cigarette smoking.

Exclusion criteria:

- Periodontal therapy was completed six months before the research.
- Medications taken six months before the research, including antibiotics, cyclosporine, phenytoin, and ongoing anti-inflammatory drugs.
- Periapical Lesion.
- Systemic alterations or an active infectious disease (diabetes, heart disease, hepatitis).
- Being pregnant or breastfeeding.

Systemic health conditions were self-reported by the participants during structured interviews.

Sample size calculation

Sample size calculation was performed adopting a minimum difference expected between groups of 1 mm and a standard deviation of 1,5 mm for clinical attachment level, considering $\alpha = 0.05$ and 80% power. A minimum sample size of 16 participants was chosen for each group, and the sample size was increased by 20% because of the potential risk of patient loss, including 19 patients in each group.

Randomization

Using a computer-generated list created a priori (SAS 9.3 software; SAS Institute Inc., Cary, NC, USA) by a team member not participating in the clinical procedures, patients were randomly assigned to one of the therapy methods:

Placebo (*n* = 19) - PSP treated with FMUD and SA of placebo for 180 days; **RSV (*n* = 19) –** PSP treated with FMUD and SA of RSV (trans-resveratrol −500 mg/day- EVOLVA (Reinach; Switzerland) for 180 days [33–35]. PL was composed of pharmaceutical talc, used for RSV encapsulation, an excipient, and an inactive medicine component.

Non-Surgical treatment

After supragingival therapy, exodontia, provisional restorations, and instructional supragingival plaque control, patients received FMUD (one session using an ultrasonic scaler with subgingival tips) [36]. Afterward, the patients were allocated to each group and enrolled in a periodontal maintenance plan (every 3 months).

Compliance monitoring methods and adverse effects evaluation

Pill counts and monthly patient interviews were used to assess medication intake compliance. All patients from both groups adhered to medication intake. Patients were followed regarding adverse effects and were asked every follow-up visit about any occurrence during systemic treatment, such as gastrointestinal discomfort or interference in daily life.

Clinical parameters

A manual probe was used to evaluate clinical measurements at baseline, three, six, and twelve-month follow-up visits. The position of the gingival margin (PGM) is the distance between the cementoenamel junction and the gingival margin; Clinical attachment level (CAL) is between the cementoenamel junction and the periodontal pocket bottom; and pocket depth (PD) is the result of subtracting PGM from CAL. The full mouth plaque (FMPS) [37] and bleeding (FMBS) [38] scores were also calculated. All measurements of clinical parameters were carried out by the same examiner (VHL), who was calibrated and masked to the therapy. Fifteen non-study participants were chosen to carry out the intra-examiner calibration. For this, the examiner measured CAL in these participants twice in 24 h. The intraclass correlation was calculated as 95% reproducibility.

Microbiologic evaluation

At baseline, 3- and 6-month follow-ups, a subgingival biofilm sample was obtained from each site and classified as deep (PD≥7) and moderate (≥4 PD≤6) using real-time polymerase chain reaction (PCR). As previously mentioned, all samples were gathered by a single, blinded, and calibrated examiner (VHL). *Tannerella forsythia (Tf)*, *Porphyromonas gingivalis (Pg)*, *and Aggregatibacter actinomycetemcomitans (Aa)* absolute quantification were measured using microbiological assays, primers, and reaction templates prepared as previously described [39].

Cytokine profile assessment using multiplexed bead assay (Luminex)

As previously mentioned [39], gingival crevicular fluid (GCF) was obtained at baseline, 3, 6, and 12-month follow-ups from moderate (≥4 PD≤6) and deep (PD≥7) sites by the same blinded examiner (VHL). Briefly,

the area was isolated and gently dried. GCF was collected by placing filter paper strips (PerioPaper, Oraflow, Plainview, NY) into the pocket until the examiner perceived a slight resistance and then leaving them in place for 15 s. The fluid volume was measured with a calibrated electronic GCF measuring device (Periotron 8000, Oraflow). After volume measurements were taken, the strips were placed into sterile tubes containing 400 mL phosphate-buffered saline (PBS) with 0.05% Tween 20. GCF samples were immediately stored at −20 °C. Using a high-sensitivity human cytokine (HTH17MAG) and multiplexing device (MAGpixTM instrument - MiraiBio, Alameda, CA, USA), the levels of interleukin (IL) −10, IL-17, IL-1β, IL-33, IL-21, IL-4, IL-23, IL-6, interferon-γ (IFN-γ), and tumor necrosis factor (TNF)-α in the GCF were measured. The standard curve range used for each marker measurement was: IL-10, 1–5000 pg/mL; IL-17, 12–50,000 pg/mL; IL-33, 6−5–20,000 pg/mL; IL-21, 5–20,000 pg/mL; IL-4, 24–100,000 pg/mL; IL-23, 336–1,500,000 pg/mL; IL-6, 3–10,000 pg/mL.

Data analysis

The primary outcome variable was CAL. Secondary outcomes embraced the additional clinical parameters, cytokine concentrations, and microbiological levels. Exploratory analyses were conducted using Box-plot and quantile-quantile (Q-Q) plots, the Shapiro-Wilk test, and the Brown-Forsythe test. A Box-Cox transformation was applied since none of the variables followed a normal distribution. For the variables PPD, CAL, and PGM, a logarithmic transformation ($\lambda = 0$) was identified as necessary. After transformation, these variables were analyzed using the Restricted Maximum Likelihood (REML) method. However, the variables FMPS and FMBS did not exhibit normality even after transformation. Therefore, Generalized Estimation Equations (GEE) were applied, which is an extension of the generalized linear model (GLM), with a gamma distribution and an AR (1) correlation matrix, evaluated through the QIC criterion. Qui-square Test was used to analyze patients'

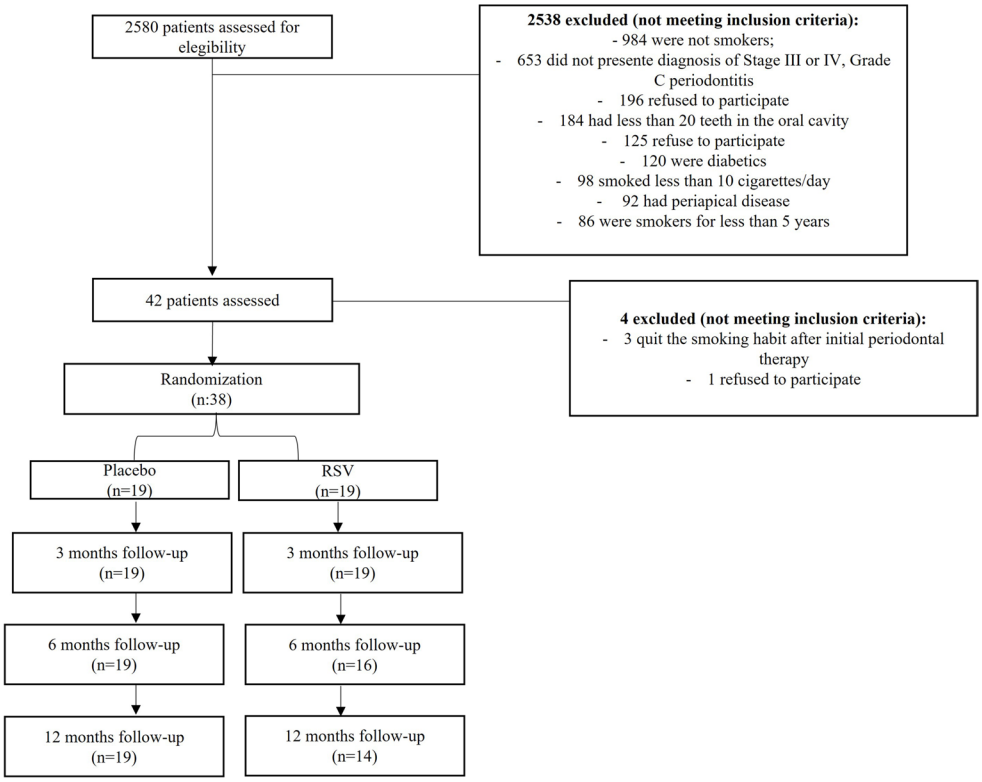

Fig. 1 Flowchart of the study. Approximately 2580 patients were screened, and 42 were included in this trial. Three of them quit smoking after receiving initial periodontal therapy, one declined to participate, and the remaining 38 were assigned at random to one of the treatment groups. During the follow-up, three patients from the RSV group quit the smoking habit and two moved to another city 2538 excluded (not meeting inclusion criteria): −984 were not smokers; −653 did not presente diagnosis of Stage III or IV, Grade C periodontitis −196 refused to participate −184 had less than 20 teeth in the oral cavity −125 refuse to participate −120 were diabetics −98 smoked less than 10 cigarettes/day −92 had periapical disease −86 were smokers for less than 5 years

socio-demographic and clinical characteristics. Finally, inter- and intra-group differences were assessed with the Mann-Whitney and Friedman tests for microbiological and immunological variables, which also did not exhibit normality even after transformation. The imputations performed showed that there was a good fit. In the sensitivity analysis with the complete model, it was observed that the results remained similar. Thus, despite the loss of follow-up, the treatment effect was not affected due to the correction by the multiple imputation method, which was computed in a grouped way in the analysis according to Rubin's rule. Every statistical analysis was conducted at an experimental significance level of 5%. SAS and SPSS software were used for all analysis.

Table 1 Patients' characteristics at baseline

Parameters	Group	
	RSV (19)	*Placebo* (19)
Age	48,64 (8.0)	50,05 (8.5)
Gender (F/M)	8/8	9/7
Race (Leuco/Melanoderma)	8/8	7/9
FMPS (%)	68.3 (30.3)	72.7 (24.2)
FMBS (%)	75.0 (17.2)	71.2 (21.0)
PGM (mm)	2.13 (0.75)	2.42 (0.53)
PD (mm)	3.61 (0.69)	3.59 (0.68)
CAL (mm)	4.31 (0.97)	4.83 (1.18)
Cigarettes/day (number - mean – SD)	16.04 (5.30)	16.26 (4.82)
Smoking duration (years – mean – SD)	10 (4.04)	11 (4.62)

Gender (female/male) and Race (leucoderma/melanoderma) were expressed as absolute numbers. The remaining parameters were presented as mean ± standard deviation. Gender and race parameters were evaluated using Qui-square ($\alpha = 0.05$). The other parameters were assessed by Student's t-test ($\alpha = 0.05$). F: female; M: male; Leuco: leucoderma; periodontal probing depth; CAL, clinical attachment level; PGM, position of the gingival margin; FMPS, full mouth plaque score; FMBS, full mouth bleeding score

Results

Clinical outcomes

Approximately 2580 patients were screened, and 42 were included in this trial. Three of them quit smoking after receiving initial periodontal therapy, one declined to participate, and the remaining 38 were assigned at random to one of the treatment groups. During the follow-up, three patients from the RSV group quit the smoking habit, and two moved to another city (Fig. 1). The groups showed homogeneity regarding age, gender distribution, and race ($p > 0.05$). In addition, intergroup analyses showed no difference for the clinical parameters evaluated ($p > 0.05$) (Table 1).

The study hypothesized that systemic administration of resveratrol as an adjunct to non-surgical periodontal therapy in smokers would promote improvements in clinical outcomes, modulate inflammatory markers, and reduce periodontopathogenic burden. Supporting this hypothesis,

patients in the RSV group demonstrated superior clinical outcomes compared to the placebo group.

Regarding probing pocket depth (PPD), the RSV group exhibited significantly lower mean values than the placebo group at 3-, 6-, and 12-month follow-ups ($p<0.05$). Within-group analyses revealed significant reductions in PPD from baseline to subsequent time points in both groups ($p<0.05$), with no additional intra-group changes observed beyond 3 months ($p>0.05$; Table 2).

For clinical attachment level (CAL), the RSV group consistently showed better outcomes than the placebo group at 3-, 6-, and 12-month evaluations, with differences of 10%, 6%, and 12%, respectively ($p<0.05$). However, no significant changes in CAL were observed within each group over time ($p>0.05$; Table 2).

Plaque gingival margin (PGM) scores were significantly lower in the RSV group across all time points ($p<0.05$), although no significant changes were detected within groups over time ($p>0.05$). At the 6-month follow-up, the full-mouth plaque score (FMPS) was 30% higher in the RSV group than in the placebo group ($p<0.05$), despite both groups presenting the highest FMPS values at baseline ($p<0.05$). After 3 months, FMPS values stabilized and remained similar between the groups throughout the remaining follow-up ($p>0.05$).

No statistically significant differences were observed between groups regarding the full-mouth bleeding score (FMBS; $p>0.05$). Nevertheless, both groups showed significant reductions in FMBS when comparing baseline with all subsequent time points ($p<0.05$), with no further changes observed between later time points ($p>0.05$; Table 2).

Table 2 Mean (mm) - SD of clinical parameters evaluated at baseline, 3, 6, and 12 months

Clinical Parameter/ Group		Baseline	3 Months	6 Months	12 Months
PD	RSV	3,61 (0,69) Aa	2,96 (0,41) Bb	2,85 (0,40) Bb	2,80 (0,35) Bb
	Placebo	3,59 (0,68) Aa	3,22 (0,51) Ab	3,07 (0,42) Ab	3,02 (0,42) Ab
CAL	RSV	4,31 (0,97) Aa	4,02 (0,90) Ba	4,04 (0,81) Ba	3,87 (0,78) Ba
	Placebo	4,83 (1,18) Aa	4,43 (0,99) Aa	4,24 (0,89) Aa	4,39 (0,93) Aa
PGM	RSV	2,13 (0,75) Aa	2,20 (0,56) Ba	2,28 (1,14) Ba	2,32 (3,27) Ba
	Placebo	2,42 (0,53) Aa	2,50 (0,50) Aa	2,53 (0,45) Aa	2,67 (0,46) Aa
FMPS	RSV	68,3 (30,3) Aa	33,8 (27,6) Ab	38,8 (22,0) Ab	28,1 (26,1) Ab
	Placebo	72,7 (24,2) Aa	36,5 (19,2) Ab	26,0 (20,3) Bb	21,2 (13,1) Ab
FMBS	RSV	75,0 (17,2) Aa	58,1 (21,0) Ab	52,7 (22,7) Ab	41,4 (26,9) Ab
	Placebo	71,2 (21,0) Aa	53,6 (20,7) Ab	50,9 (18,9) Ab	42,5 (20,4) Ab

Capital different letters indicate differences between groups, $p<0.05$; Lower case different letters indicate intra-group differences, $p<0.05$. PPD, periodontal probing depth; CAL, clinical attachment level; PGM, position of the gingival margin (REML method, $\alpha=0.05$). FMPS, full mouth plaque score; FMBS, full mouth bleeding score (GLM method, $\alpha=0.05$)

Microbiological results

In the analysis of *Aa*, the results indicated a significant difference ($p<0.05$) for a higher concentration of Aa in the Placebo group at 3 months when moderate and deep periodontal sites were evaluated. At 6 months, only in deep sites, the results demonstrated a significant difference ($p<0.05$) for a greater amount of Aa in the Placebo group. No statistically significant difference was found for moderate and deep sites in any evaluated periods ($p>0.05$). There were no intra-group and inter-group differences for the Tf pathogen at all periodontal pocket depths over time ($p>0.05$). Considering the Pg pathogen, the PL group presented a higher number of this pathogen at baseline ($p<0.05$). Regarding the intra-group comparison, a difference in the amount of Pg for the PL group in moderate sites at 3 and 6 months was observed when compared with the baseline ($p<0.05$), but with no difference between 3 and 6 months ($p>0.05$). There was no difference in intergroup analysis ($p>0.05$) (Table 3).

Cytokine results

At 3 months, the RSV group showed significantly lower IL-1β levels at deep sites compared to the Placebo group ($p<0.05$). IL-6 concentrations were also significantly reduced in the RSV group at both 3 and 12 months in moderate and deep sites ($p<0.05$). No significant intergroup differences were observed for the other inflammatory markers ($p>0.05$). These findings are described in Table 4.

Discussion

Smoking is a risk factor for periodontitis and affects the response of patients to periodontal therapy [1, 2, 40–42]. The positive effect of RSV reducing the progression of experimental periodontitis has been shown in animal models, including healthy and systemically compromised

Table 3 Amount (log10 _ SEM) of *Aggregatibacter actinomycetemcomitans (Aa)*, *Tannerella forsythia (Tf)* and *Porphyromonas gingivalis (Pg)* and at baseline, 3- and 6-months post-therapy

Aa	Moderate		Deep	
	RSV	PL	RSV	Placebo
Baseline	1.64 ± 1.01	1.72 ± 1.35	1.77 ± 0.96	1.82 ± 0.88
3M	1.61 ± 1.02	2.29 ± 1.10 *	1.73 ± 0.90	2.39 ± 1.14 *
6M	1.99 ± 0.92	2.35 ± 0.87	1.77 ± 0.94	2.23 ± 1.08 *
Tf				
	RSV	**Placebo**	**RSV**	**Placebo**
Baseline	3.43 ± 1.37	3.29 ± 2.36	3.55 ± 1.66	3.39 ± 2.21
3M	2.65 ± 1.59	2.55 ± 1.87	2.94 ± 1.52	3.70 ± 1.65
6M	2.85 ± 1.66	2.63 ± 1.69	2.98 ± 1.48	3.31 ± 1.57
Pg				
	RSV	**Placebo**	**RSV**	**Placebo**
Baseline	1.26 ± 1.65	2.08 ± 1.91	0.39 ± 1.11	1.09 ± 1.46 *
3M	0.36 ± 0.99	0.69 ± 1.51 †	0.76 ± 1.59	0.86 ± 1.67
6M	0.88 ± 1.57	0.55 ± 1.35 †	1.24 ± 1.85	0.63 ± 1.52

* Indicates differences between groups (Mann-Whitney Test; $p < 0.05$)

† Indicates intra-group differences from baseline (Friedman; $p < 0.05$)

animals. Clinical studies also demonstrated a good impact of RSV associated with non-surgical periodontal treatment of healthy and diabetic patients, with modulation of immunological markers [29–31] and improvement of clinical parameters [29]. For the first time, the effect of systemic treatment with resveratrol as an adjuvant to non-surgical periodontal therapy through clinical, inflammatory, and microbiological analysis was examined.

Overall, the RSV group demonstrated improved clinical outcomes, including lower PD, CAL, and PGM, along with reduced IL-1β levels at 3 months, decreased IL-6 levels at 3 and 6 months, and a reduction in *A. actinomycetemcomitans* counts at 6 months in deep sites.

In this trial, improvement in the clinical attachment level was observed with RSV treatment. PD, CAL, and PGM were also lower in the RSV group. These results are consistent with other RSV studies, in which better clinical parameters were found after systemic treatment with RSV adjunct therapy than isolated non-surgical periodontal therapy [29]. Interestingly, Zhang et al. (2022) [30] treated periodontitis

Table 4 Mean (SD) of concentrations (pg/µl) of mediators for both groups at baseline, 3. 6-, and 12-months post-therapy

Marker	Moderate sites				Deep sites			
IFN-γ	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
RSV	1.27 (1.07)	2.83 (2.60)	4.11 (2.92) †	4.59 (2.78) †‡	0.95 (0.90)	3.13 (2.87) †	2.68 (2.30) †	2.35 (2.09)†
PL	1.49 (1.49)	4.073 (0.38) †	4.61 (2.73) †	4.78 (2.59) †	0.89 (1.20)	3.01 (2.43) †	3.10 (2.83) †	2.96 (0.47) †
IL-10								
RSV	0.29 (0.26)	0.62 (0.24)	1.64 (1.78) †	1.19 (0.69) †‡	0.20 (0.18)	0.53 (0.16)	0.76 (0.76) †	0.99 (0.98) †‡
PL	0.40 (0.63)	0.75 (0.68)	0.99 (0.67) †	1.13 (0.52)	0.26 (0.38)	0.46 (0.28)	0.44 (0.36)	0.53 (0.22)
IL-17								
RSV	1.22 (0.96)	2.78 (2.39)	4.17 (2.76) †	4.12 (2.85) †	1.14 (1.15)	2.68 (2.18)	2.70 (2.29) †	2.30 (2.17)
PL	1.14 (0.99)	3.67 (2.91)	4.51 (3.07)	6.43 (3.56)	1.02 (1.68)	2.37 (1.92)	2.48 (2.66)	2.73 (1.25)
IL-1β								
RSV	97.13 (114.61)	103.7 (102.4)	98.48 (82.65)	43.78 (42.26)	28.78 (49.63)	35.36 (52.92)	48.38 (70.06)	76.06 (74.11)
PL	71.49 (58.84)	94.08 (66.8)	73.48 (74.81)	98.79 (46.08)	36.21 (34.17)	92.6 (84.2)	43.97(33.21)	88.13 (75.45)
IL-33								
RSV	0.55 (0.51)	1.29 (1.25)	2.12 (2.41) †	2.26 (2.45) †	0.34 (0.36)	1.21 (1.17)	1.25 (1.15) †	1.32 (1.15) †
PL	0.60 (0.43) A	1.64 (1.54)	1.90 (1.49) †	1.97 (1.52) †	0.27 (0.16)	0.90 (0.74) †	1.03 (1.15) †	1.09 (1.16) †
IL-21								
RSV	1.32 (1.09)	4.03 (4.20)	6.04 (5.42) †	6.50 (5.44) †	0.83 (0.63)	2.54 (2.16) †	2.33 (2.51)	2.50 (2.56) †
PL	1.45 (1.26)	4.18 (3.85)	6.13 (4.09) †	6.46 (4.05) †	0.86 (0.50)	2.77 (2.15)	2.42 (1.95)	2.56 (1.96)
IL-4								
RSV	0.013 (0.019)	0.020 (0.023)	0.051 (0.065) †	0.016 (0.012)	0.006 (0.008)	0.014 (0.012)	0.019 (0.023) †	0.013 (0.010)
PL	0.016 (0.019)	0.024 (0.040)	0.047 (0.060) †	0.028 (0.019)	0.008 (0.016)	0.018 (0.016) †	0.020 (0.020) †	0.015 (0.007)
IL-23								
RSV	0.11 (0.16)	0.19 (0.19)	0.27 (0.25) †	0.07 (0.05) $	0.05 (0.06)	0.15 (0.14) †	0.15 (0.14) †	0.04 (0.04)
PL	0.08 (0.08)	0.23 (0.26)	0.29 (0.20) †	0.08 (0.05) $	0.04 (0.04)	0.12 (0.13) †	0.16 (0.18) †	0.05 (0.04)
IL-6								
RSV	2.07 (1.85)	4.67 (4.20)	6.61 (5.31) †	5.33 (4.14) †	1.23 (1.55)	3.57 (3.73)	5.06 (5.37) †	2.10 (0.89) †
PL	2.63 (1.69)	8.11(9.50) *	8.15 (5.81)	8.01 (3.52) *	1.84 (1.63)	4.69 (3.06) *†	4.44 (4.22) †	3.50 (2.67) *
TNF-α								
RSV	0.84 (0.67)	2.03 (1.76) †	2.55 (2.04) †	2.98 (2.64) †	0.50 (0.44)	1.39 (1.11)	1.79 (1.73)	3.18 (3.07) †‡
PL	0.71 (0.45)	2.28 (1.90) †	2.37 (1.24) †	3.36 (1.58) †‡	0.55 (0.39)	1.40 (1.10)	1.09 (0.86)	3.21 (3.23) †‡

* Indicates differences between groups (Mann-Whitney Test; $p < 0.05$); †Indicates intra-group differences from baseline (Friedman; $p < 0.05$). ‡ Statistically significant difference from 3 months (Friedman; $p < 0.05$); $ Statistically significant difference from 6 months (Friedman; $p < 0.05$)

with RSV alone and saw improvements in clinical and inflammatory parameters with RSV treatment without any adverse effects. On the other hand, Nikniaz et al. (2023) [31] and Javid et al. (2019) [29] did not observe any clinical improvements with RSV administration adjunctive to non-surgical periodontal therapy. It is worth saying that in the Javid et al. study, the included population was type 2 diabetic, and the time of administration of RSV and follow-up was only 4 weeks (480 mg/day of RSV). Besides, the authors used resveratrol-enriched Polygonum cuspidatum extract instead of pure RSV, which was used in the present study, meaning we don't know the exact amount of RSV added. Furthermore, although Nikniaz et al. [31] included systemically healthy patients, the time of RSV treatment (480 mg/day) and follow-up were also 4 weeks. In the present study, a dose of 500 mg of highly pure resveratrol (>98%) was used for a substantially more extended period; 6 months. This dosage regime probably allowed us to see the beneficial effects of resveratrol, even in smokers.

PD, CAL, and PGM were lower in the RSV group. RSV's anti-inflammatory, antioxidant, and bone-preserving effects might explain this effect on clinical parameters. These outcomes are also in line with previous studies in animal models that showed a reduction of periodontal breakdown with RSV treatment in systemically healthy animals [21, 22] and in diabetic [27], arthritic [24], and animals exposed to smoke [23, 25]. These studies demonstrated reduced alveolar bone loss through modulation of inflammatory markers, such as IL-17, IFN-γ, IL-1β, IL-6, TH17/TH2 response, and IL-4. The development of periodontitis is largely influenced by the increase in pro-inflammatory marker levels (IL-17, IFN-γ, IL-1β, IL-6, TH17/TH2 response) and the decrease in anti-inflammatory marker levels [43]. In line with these results, this study showed that RSV downregulated IL-1β at a 3-month time-point in deep sites and IL-6 at 3 and 12 months in moderate and deep sites.

It is well known that smoking status is one of the more important epigenetic factors that increase the risk for periodontitis development (as well as increasing its severity) [44]. Moreover, smoking obtunds the effects of various therapies used for treatment of periodontitis [45, 46]. Smoking and the components of smoke (and in our estimation the aryl hydrocarbons are likely amongst the most important pathogenic factors) are linked to the alteration of immunological responses by reducing neutrophil function [47, 48], altering Th1/Th2/Th17 immune homeostasis [49, 50], and activation of nuclear factor kappa B (NFκB) and mitogen-activated protein kinase (MAPK) [28, 51]. To reemphasize a previous point, products of combustion found in cigarette smoke, such as aryl hydrocarbons, such as benzo a pyrene or dimethylbenzanthracene [52], inhibit osteoblast proliferation and differentiation, which is reversed by resveratrol [53] while also upregulating the synthesis of ROS [53], which stimulates more inflammation. In this context, RSV seems to be an interesting bioactive product due to its biological properties regarding the anti-inflammatory [21–24] and antioxidant effect [25–27] by modulation of immune-inflammatory and stress oxidative markers and to the specific action as an antagonist of the aryl hydrocarbon receptor [53–56].

It is well known that nicotine/cotinine has important vasoconstrictor effects on periodontal blood vessels, reducing the gingival index and bleeding on probing parameters in smoking patients [57, 58]. Furthermore, long-term outcomes of patients with maintained and treated periodontitis were seen in longitudinal investigations, which demonstrated that bleeding on probing was not a reliable indicator of the disease's progression [59]. Therefore, the absence of differences between the groups regarding FMBS might be a consequence of smoking habits in both groups, instead of the lack of RSV effect in this parameter.

Beyond its recognized host-modulatory properties, resveratrol also demonstrated microbiological effects in this study. Although RSV is not a conventional antimicrobial agent, its impact on the subgingival microbiota may occur through both indirect and direct mechanisms. Indirectly, RSV could modulate the microbial environment by altering the inflammatory and oxidative milieu within periodontal tissues. However, in vitro studies have shown that RSV exerts mild bacteriostatic activity and can potentiate the efficacy of conventional antibiotics, suggesting a possible direct antimicrobial effect [60, 61].

Importantly, smoking has been shown to induce a dysbiotic shift in the periodontal microbiome, characterized by an increase in pathogenic species and a reduction in microbial diversity and resilience [62, 63]. Studies consistently report that smokers harbor higher levels of *A. actinomycetemcomitans* and *P. intermedia* compared to non-smokers [64, 65]. In the present trial, a significant reduction in *A. actinomycetemcomitans* counts was observed in the RSV group at 6 months, suggesting a microbiological benefit that may contribute to the improved clinical outcomes seen. Whether this effect is mediated by direct antimicrobial action or secondary to host modulation remains to be elucidated, but the finding supports the hypothesis that RSV may help counteract the microbial imbalances associated with smoking.

Additionally, RSV has been shown to inhibit *P. gingivalis* LPS-induced monocyte adhesion to the endothelium in vitro, suggesting a potential role in attenuating pathogen-induced vascular inflammation within periodontal tissues [66]. This dual capacity, modulating both host response and microbial composition, reinforces the therapeutic potential of RSV in periodontal disease, particularly in systemically compromised or high-risk populations such as smokers.

It is worth noting, however, that not all studies have demonstrated a microbiological effect of RSV. For instance, Cirano et al. (2016) [66] reported no significant changes in pathogen levels in an animal model treated with RSV. The discrepancy may stem from differences in experimental design, as their study evaluated microbial dynamics during biofilm formation in vivo, whereas the current clinical trial assessed established subgingival microbiota following non-surgical therapy.

Additionally, this trial found significantly lower IL-1β levels at 3 months in deep sites in the RSV group, as well as lower IL-6 concentrations at 3 and 12 months in both moderate and deep sites ($p < 0.05$). Smoking is well known to alter the host's immunoinflammatory response to pathogenic stimuli. Considering that IL-1β plays a pivotal role in regulating multiple pro-inflammatory cytokines, including TNF-α and IL-6, and that IL-6 exerts pleiotropic effects as a key acute-phase cytokine, these mediators may provide more objective and mechanistic evidence for clarifying the pathogenesis linking smoking to periodontal disease. Supporting this, animal studies have demonstrated elevated levels of IL-1β and Th1/Th2 cytokines in experimental periodontitis rat models exposed to cigarette smoke compared with non-exposed controls [23]. Notably, the same research group observed higher IL-1β, IL-6, and Th1/Th2 levels in smoking-exposed animals even in the absence of induced periodontitis [23]. Clinical studies similarly report higher concentrations of IL-1β and IL-6 in the gingival crevicular fluid of smokers compared with non-smokers [67, 68]. Consistently, another study found significant associations between smoking and increased total gingival crevicular fluid IL-6 levels [69].

This study has some limitations that should be considered when interpreting the findings. The relatively small sample size may limit the generalizability of the results. Although the trial was adequately powered to detect differences in the primary outcomes, larger multicenter studies are needed to validate and expand these findings across more diverse populations. Another limitation is the absence of salivary cytokine assessment. While gingival crevicular fluid (GCF) was selected for its high sensitivity in reflecting site-specific periodontal inflammation, combining GCF and saliva analyses could have provided a broader overview of both localized and systemic inflammatory responses to RSV, particularly in smokers. In addition, site-specific probing depth changes were not analyzed, which might have offered a more detailed understanding of treatment effects at individual sites. The systemic health status of participants was also self-reported, which may introduce bias; future studies should confirm comorbidities through medical evaluation, given the well-established bidirectional links between periodontal and systemic health.

It is also important to note that all participants received structured supportive periodontal therapy (SPT) every three months, following current clinical guidelines and supported by evidence for maintaining periodontal stability in high-risk groups [70, 71]. This standardized regimen likely contributed to the favorable clinical outcomes in both groups and should be taken into account when comparing these results with studies employing different maintenance protocols.

Despite these limitations, the consistent clinical improvements observed—alongside reductions in pro-inflammatory cytokines—support the potential role of RSV as an adjunctive therapy in smokers with periodontitis. Within the limits of this study, it can be concluded that the use of RSV in combination with non-surgical periodontal therapy improved clinical, immunological, and microbiological parameters in smoking patients. Therefore, RSV emerges as a promising and safe bioactive agent for adjunctive use in periodontal therapy, particularly in this high-risk population.

Author contributions Vanessa Haguihara Luchesi – Investigation (Worked at experimental phase - clinical procedures and patient recruitment);Ana Paula Oliveira Giorgetti - – Investigation (Worked at experimental phase - clinical procedures and patient recruitment);Mônica Grazieli Corrêa - Data Curation; Writing – original draft preparation (Analysis, and interpretation of data for the work; Laboratorial analysis; Drafting the work); Project Administration;Vanessa Gallego Arias Pecorari – Formal Analysis (Statistical analysis of the data); Validation;Bruno Braga Benatti – Conceptualization; Methodology (Substantial contributions to the conception or design of the work);Howard C. Tenenbaum – Conceptualization; Writing - Review &; Editing (Substantial contributions to the conception and design of the work; Drafting the work and revising it critically for important intellectual content);Fabiano Ribeiro Cirano - Conceptualization; Supervision (Substantial contributions to the conception or design of the work);Suzana Peres Pimentel – Conceptualization; Supervision (Substantial contributions to the conception or design of the work);Marcio Zaffalon Casati - Conceptualization; Writing - Review &; Editing; Funding acquisition; Resources; Project Administration (Substantial contributions to the conception and design of the work; Revising the work critically for important intellectual content; Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved);

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Data availability No datasets were generated or analysed during the current study.

Declarations

Compliance with ethical standards Disclosure of potential conflicts of interest: The authors report no conflicts of interest related to this study.

Informed consent This study was a parallel, double-blinded, randomized clinical trial (Rebec identifier: RBR-3gt65c), and performed in compliance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines. The study was approved by the review board of the university (Ethics Committee of Paulista University − 1.542.367) and was carried out in compliance with the 2013 revision of the 1975 Helsinki Declaration. The included patients gave their informed and written consent.

Competing interests The authors declare no competing interests.

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